Anopheles (Cellia) takasagoensis Morishita 1946, an additional species in the Balabacensis Complex of Southeast Asia (Diptera: Culicidae)[1]

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ABSTRACT. *Anopheles (Cellia) takasagoensis* Morishita, a member of the Balabacensis Complex from Taiwan, is removed from synonymy with *An. (Cel.) balabacensis* Baisas, elevated to specific status and described in the adult, pupal and larval stages. The bionomics and medical significance of this species are reviewed.

INTRODUCTION

In the course of studying the Leucosphyrus Group of the Oriental region for the past 3 years, we have made a number of significant findings concerning the identity of various geographic populations of the nominal species *An. (Cel.) balabacensis* Baisas, one of several members of the Leucosphyrus Group as defined by Colless (1956) and Reid (1968). Through a multidisciplinary approach, which has included morphology, biology, cross-matings and cytogenetics, it has become clear that what has been widely recognized as a single, highly variable, geographically widespread species, *balabacensis*, actually represents a species complex composed of several morphologically similar, yet genetically isolated, species; the exact number of species involved in this complex presently is uncertain. Currently recognized members of the complex include: *balabacensis balabacensis*, *b. baisasi* Colless, *b. introlatus* Colless, *dirus* Peyton and Harrison and *takasagoensis*. Studies are continuing in our respective organizations to further elucidate these taxa. In our first report concerning this complex, we described *An. (Cel.) dirus* Peyton and Harrison (1979) from Thailand. The present paper deals with the morphological description of the Taiwan species *takasagoensis* Morishita. Separate reports on laboratory hybridization and cytogenetic studies concerning *takasagoensis* will appear later.

Regrettably, these findings will make the recognition of several species in the Balabacensis Complex, if based only on morphological characters, extremely difficult. All species in this complex are very similar in

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appearance, and there is considerable intraspecific morphological variation in all life stages, particularly in the adult stage. Most are recognizable in at least one life stage; however, adult specimens with associated pupal and/or larval exuviae provide the best means for accurately identifying the various taxa. Identifications based on specimens without associated stages will inevitably lead to some misidentifications, especially where these species occur sympatrically. This should not be a major problem with *takasagoensis* since it appears to be endemic to Taiwan and the only member of the Leucosphyrus Group present on the island.

Since several species of the Leucosphyrus Group are demonstrated vectors of human and/or simian malaria parasites, the recognition of these additional taxa will significantly alter the present concept of malaria epidemiology in a number of areas of Southeast Asia. In areas where 2 or more of these species are sympatric, their distributions and vector status will need reinvestigation. Additional information in this regard will be provided in a future revision of the Leucosphyrus Group.

Anopheles (Cel.) takasagoensis is apparently a fairly uncommon species, occurring principally in the less accessible low hill and mountainous regions of the island, with most collections coming from the central and southern regions. High numbers of adults are occasionally encountered in some of these areas (Chuang et al. 1965, 1967). Very little attention has been given to this species, and until recently it has been treated by most workers as *leucosphyrus*. Koidzumi (1925:318) was apparently the first to report collecting in Taiwan, a species "that resembled *leucosphyrus* Dönitz but differed from it." Matumoto and Motoura (1939) described the adult and larva of this species as *leucosphyrus*. These appear to be the only references to the species until Morishita (1946) redescribed it as *leucosphyrus* var. *takasagoensis*. Morishita republished this article in 1949, but with a different title and in another journal. Chow (1949) continued to list the species as *leucosphyrus*. Colless (1956, 1957) was apparently unaware of Morishita's articles since neither the name nor the titles appeared in his revision of the Leucosphyrus Group. However, Colless suggested in a footnote (1956:55) that specimens examined from Taiwan were typical of *balabacensis* Baisas. Stone et al. (1959) also did not include a reference to *takasagoensis* in their catalog of the Culicidae. Later, in correspondence (8 August 1961) with Stone, Colless stated that he considered var. *takasagoensis* a synonym of *balabacensis*, and Stone (1963:119) referenced the article for the first time and listed *takasagoensis* as a synonym of *balabacensis*.

During our study we have examined a moderate number of feral adults and larvae, and numerous specimens of all stages from the F_{81} generation of a colony established in the Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. This colony was originally established and maintained in Taiwan from material collected at Peiyuan village, Tungho township, Taitung county, Taiwan, in 1972 by J. C. Lien. Morphologically, *takasagoensis* is very similar to *balabacensis* and *dirus*, but we have found small, fairly consistent differences in the adult, pupal and larval stages that are sufficient to separate the former from both species. Morphologically, it is closest to *dirus* and differs from *balabacensis* in several of the characters used in Peyton and Harrison (1979) to separate *dirus* from *balabacensis*.

In addition to morphological evidence, reciprocal cross-mating experiments between *dirus* and *takasagoensis* provided biological and cytogenetic evidence to support the elevation of *takasagoensis* to specific status. These experiments, conducted at AFRIMS, Bangkok, involved the Bangkok (Chonburi) strains of *dirus* and the Peiyuan-Taiwan strain of *takasagoensis*, the latter kindly provided through J. C. Lien and the U.S. Navy Medical Research Unit #2 (NAMRU-2), Taiwan, in February 1979. Crosses were made by the modified anopheline artificial copulation technique described by Ow-Yang et al. (1963). During these experiments *dirus* and *takasagoensis* were conclusively shown to be separate species by the following: (1) complete sterility of F_1 hybrid males in both directions, supported by abundant morphological abnormalities in the reproductive system; (2) reduced viability of adult backcross males, and non-functional and/or abnormal external genitalia in males produced in certain backcrosses utilizing F_1 hybrid females; and (3) asynapsis of large areas of F_1 hybrid fourth stage larval polytene salivary chromosomes. Additional data from these crosses will be published elsewhere.

Terminology used follows that of Harrison and Scanlon (1975) and other Medical Entomology Project studies. Descriptions of the adult and larval stages are based upon feral and colony specimens. The pupal description is based entirely upon colony specimens and may represent a limited range of variation as compared to that which may occur in natural populations. The terminology of the toothed margin index of the pupal paddle follows that of Colless (1956).

Anopheles (Cellia) takasagoensis Morishita

(Figs. 1-3)

Anopheles leucosphyrus var. *takasagoensis* Morishita 1946:21, 65 (♀, ♂, L); Stone 1963:119 (Colless *in litt.* syn. of *balabacensis* Baisas); Reid 1968: 297 (syn. of *balabacensis* Baisas).

Anopheles leucosphyrus of Koidzumi 1925:318; Matumoto and Motoura 1939:1642 (♀*, ♂*); Chow 1949:1; Chang et al. 1950:289; Chang and Huang 1954-55:60, 343 (♀*, L* key).

Anopheles leucosphyrus balabacensis of Colless 1956:55 (in part, tax.).

Anopheles balabacensis balabacensis of Colless 1957:133 (in part, tax.); Chuang et al. 1967:3; Reid 1968:297 (in part, tax.); Knight and Stone 1977: 35 (catalog).

Anopheles balabacensis takasagoensis of Chuang et al. 1965:3; Reid 1968:297 (footnote, tax.).

FEMALE (Fig. 1). *Head.* Proboscis 1.85-2.36 mm, ratio to forefemur 1.01-1.16; palpus 1.88-2.29 mm, ratio to proboscis 0.87-1.01, segments 2-4 with narrow apical white bands, segment 5 with a broad apical white band

varying from 1.57-4.00 ($\bar{X}$=2.55) length of the preapical dark band, preapical dark band 0.80-2.33 ($\bar{X}$=1.22) length of apical white band of segment 4. *Thorax.* Propleural setae 1:1-3:3, usually (38/40) 2:2. *Wing.* [Fig. 1, showing 3 examples of variations in wing spotting on costa (C), subcosta (Sc) and radius (R)]. Light and dark spots of veins M, Cu and A, highly variable in number, length and placement, spots on C, Sc, and R less variable; prehumeral (PHP), humeral (HP) and presector pale (PSP) spots of C usually prominent, occasionally PHP or HP reduced, rarely absent; presector dark (PSD) spot of R usually equal to the corresponding dark spot on C on both wings (36/48), occasionally with slight extension basally on one or both wings but usually not beyond 0.5 of PSP of C, rarely (3/48), reaching to level of humeral dark (HD) spot of C, usually with 1,2 pale interruptions, rarely (2/48) with 3 on one wing only; C without accessory sector pale (ASP) spot, Sc without ASP, rarely with 2-4 pale scales on one or both wings (3/48), ASP always present on R, usually small; dark spot basal to ASP 0.60-3.00 length of ASP, usually 1.00 or more, rarely with a small pale interruption on one wing only (3/48); middle dark (MD) spot on R_1 distal to ASP with 1-4 small pale spots, usually 2; preapical dark (PD) spot on R_1 with 1-4 small pale spots; apical dark (AD) spot on R_1 occasionally with a small pale spot or 2-4 pale scales on one or both wings (19/48); subcostal pale (SCP) spot on C, 0.80-2.25 length of preapical pale (PP) spot on C, usually longer than PP on both wings (39/48), or (45/48) on at least one wing, rarely shorter than PP on one wing only (2/48) or the two spots subequal; vein 1A with 5-8 pale spots; ratio of length of cell R_2 to R_{2+3} 1.62-1.92, ratio of length of cell R_2 to cell M_2 1.16-1.23; apical pale (AP) spot reaching to vein R_2 followed by a short dark spot between R_2 and R_3, and a longer pale spot extending slightly past R_{4+5}, pale fringe spots at veins M_{1+2}, M_{3+4}, Cu_1 and often present at Cu_2, and between Cu_2 and 1A. *Legs.* Femora, tibiae and tarsomeres 1 speckled with pale spots, tarsomeres 2 usually with 1-3 pale spots on at least one of each pair of legs; apical pale band on hindtibia without dark scales extending into basal portion; foretarsomeres 1-4 with broad apical pale bands, tarsomeres 2-4 with basal pale bands, bands more or less complete but less distinct on ventral surface, basal bands subequal to or slightly longer than apical bands, tarsomeres 4,5 occasionally predominantly pale dorsally; hindtarsomere 4 with a conspicuous basal pale band, frequently equal to length of apical pale band on tarsomere 3, tarsomere 5 occasionally with a small basal pale band, apex usually pale white or beige. *Abdomen.* Tergum VI occasionally with a very few inconspicuous, narrow dark scales on caudal margin, VII always with a few dark scales on caudal margin, VIII covered with moderately broad golden scales, occasionally with a few basal dark scales and caudomedian apical white scales; sternum VI rarely with a few scattered narrow dark scales on caudal margin, VII with a dense caudomedian patch of semi-erect dark scales, VIII with small lateral patches of creamy golden scales.

MALE (Fig. 1). Essentially as in female except for sexual characters. Wing as compared to that of female generally paler with reduced scaling, costal spots highly variable and pale spots usually much longer. Proboscis longer than female, 2.03-2.68 mm, ratio to forefemur 1.38-1.51; palpal segment 2 with a dorsal patch of pale scales, apex of segment 2 bare, giving the appearance of a pale band at the joint of segments 2,3, segment 3 with a long dorsal patch of pale scales at middle, apex with a broad pale band and

a few dark scales, dorsal surface of 4 and 5 pale except for dark basal band on each segment, ventral surface of 4 and 5 pale except for dark basal band on each segment and ventrolateral line of dark scales usually extending from base of 4 to near apex of 5. *Thorax.* Propleural setae 1:1-2:2, usually 2:2. *Wing.* PSD spot on vein R with 0-3 pale interruptions, most commonly 1 (13/17); C occasionally with ASP on one or both wings (4/17), Sc more often with a distinct ASP spot or with a few pale scales (10/17); SCP and PP of costa variable in length, usually both noticeably longer than on female and either may be longer than the other; PD of R_1 with 1-3 pale spots; AD of R_1 rarely with a small pale spot. *Legs.* Foretarsomeres 1-4 usually with apical bands only, 4,5 occasionally entirely pale dorsally. *Abdomen.* As compared to female, dark scales on tergum VII more numerous and sternum VII with fewer scales, sternum VIII profusely covered with snowy white scales, basimeres of genitalia clothed with similar scales on sternal and lateral surfaces, with a few dark brown scales basolaterally.

PUPA (Fig. 2). Modal condition of chaetotaxy as figured. Diagnostic features as follows: *Abdomen.* Seta 1-III with 5-11 branches, 5-III 8-14 branched; 1-IV with 3-6 branches, 5-IV with 5-9 branches, 9-IV short, length 0.029-0.061 mm ($\bar{X}$=0.043), ratios of length of seta 9, IV/III 1.33-3.08 ($\bar{X}$=2.22), IV/V 0.29-0.54 ($\bar{X}$=0.042), 1-V usually single (29/32), occasionally bifurcated apically or rarely (3/32) in basal 0.5, 5-V with 4-7 branches; 1-VI single, rarely bifurcated apically, 5-VI with 3-7 branches; 1-VII single, 5-VII with 2-6 branches; toothed margin index of paddle 0.70-0.88 ($\bar{X}$=0.82).

LARVA (Fig. 2, 3). Modal condition of chaetotaxy as figured. Diagnostic features as follows: *Head.* Seta 2-C single, usually with 2-4 fine lateral barbs; 4-C posterolaterad of 2-C, single to triple, 2,3 branched on one or both sides in 17/25 specimens; distance between insertions of 2-C and 4-C (measured from mounted exuviae) wide, 0.084-0.111 mm ($\bar{X}$=0.100); length of 4-C 0.44-0.072 mm ($\bar{X}$=0.062), extending forward 0.46-0.76 ($\bar{X}$=0.63) the distance between the insertions of 2-C and 4-C; 13-C with 3-5 branches. *Thorax.* Seta 1-P with 12-17 branches, stem strong but not flattened and expanded, about equal to stem of 2-P, basal sclerotized tubercles of setae 1,2-P large, fused basally, both with a strong, apical, rounded or pointed tooth projecting forward over the base of each seta; 14-P usually with 5,6 branches, range 4-8; 14-M with 6-11 branches; 3-T with 3-7 weak branches, rarely with weakly developed leaflets. *Abdomen.* Seta 1-I small, weak, with 4-7 branches, 3-I usually single, rarely bifid, 9-I usually with 3 branches, range 3-5; 1-II weakly developed, with 9-15 branches, without distinct leaflets; 2-IV with 3-5 branches, 6-IV with 2,3 branches, 13-IV with 3,4 branches, greater than than 0.50 the length of 10-IV; 2-V usually with 4-6 branches, range 3-6; 1-VII smaller than 1-VI with 10-14 lanceolate leaflets; 1-X inserted just off the ventral border of saddle or within a distinct open notch of ventral border of saddle; pecten usually with 4-6 long teeth and 6-11 distinctly shorter teeth.

TYPE-DATA. There is no reference to a type-designation or a depository for type-specimens in Morishita (1946), therefore, it is presumed that type-specimens do not presently exist. Knight and Stone (1977) list the depository for the type as "location unknown." The type-localities for

takasagoensis are Dairi, Tamazato District, Karenko Prefecture, and Kanshirei, Kagi District, Tainan Prefecture, Taiwan.

DISTRIBUTION. Apparently *takasagoensis* is restricted to the island of Taiwan. The form treated by Teng et al. (1974) as *balabacensis* from Hainan Island is probably *dirus* Peyton and Harrison (1979).

Specimens examined: feral specimens, 21 ♀, 16 ♂, 1 p, 1 l, 17 L; colony specimens, 50 ♀, 15 ♂, 37 p, 20 l, 20 L. Collection localities from labels on specimens and published records include: *Hualien:* Karenko, Dairi, Tamazato District. *Tainan:* Kanshirei, Kagi District. *Taitung:* Peiyuan, Tungho; Ta-Wu. *Chiai:* Cho-Kou, Fian-Lu. *Taichung:* Takeng, Pei-Tung; Chi-Feng-Keng, Hsin-She; Chung-Keng-Ping, Tung-Shi. *Taipei:* Chung Ho, Hsin-She. *Pingtung:* Shimen, Mutan; Ch'oa Chow. Prefecture unknown: Hsin-Chi, Wan-Luan, Tai Pin-Tung, Hsien Valley. Specimens from collection number 01539, illustrated in figure 1, were collected in Shimen, Mutan Pingtung, Taiwan by J. C. Lien and were borrowed from the Australian National Insect collection, Canberra.

DISCUSSION. Although all of the presently recognized species of the Balabacensis Complex are extremely similar in all stages, *takasagoensis* morphologically, shows the greatest affinity to *dirus*. The wing of the adult female of *takasagoensis* differs from that of *balabacensis* primarily in the absence of an accessory sector pale spot (ASP) on veins C and Sc. In this respect, it is similar to *dirus* and most other mainland taxa of the complex. The presector dark spot (PSD) of vein R is more like that of *balabacensis* with most specimens showing no basal extension of this spot beyond the corresponding dark spot on C or, if there is a slight extension, it rarely reaches beyond the middle of PSP of C. On the wings of *takasagoensis* females the SCP spot of C is usually longer than the PP spot of C on both wings (39/48) or on at least one wing (45/48) and with only 2/48 actually shorter than PP on one wing only. In females of *dirus* on C the SCP spot is 0.44-1.50 the length of PP spot and is usually shorter than the PP spot on both wings (31/68) or on at least one wing (53/68), but 7/68 had the SCP spot longer than PP spot on both wings and 16/68 had it longer on at least one wing. A small percentage of specimens of both species have the 2 spots equal on one or both wings. This character is of little value in species identification of single specimens, but, in combination with the absence of a basal extension of the PSD spot of R, it becomes more useful and should prove to be of greater importance in recognizing different populations. Most other characters in the adult of both species are more variable with a higher degree of character overlap.

Anopheles takasagoensis is most easily distinguished from *dirus*, *balabacensis* and most other species of the Leucosphyrus Group in the pupal stage. Seta 9-IV of *takasagoensis* is of the short type and in this respect, very similar to *dirus*. Seta 1-V-VII is single in *takasagoensis*, whereas in *dirus* and *balabacensis* it is never single on all 3 segments. In *dirus* 1-V-VII is branched as follows: 1-V 2-4 branched, rarely (1/45) single, 1-VI single to 3 branched, rarely (2/45) single on both sides and occasionally (12/45) single only on one side, 1-VII single to 3 branched, occasionally (13/45) single on both sides, and when single on both sides, 1-V,VI always

branched. In *balabacensis* this seta is: 1-V 2-5 branched, rarely (1/30) double on both sides and occasionally (4/30) double on one side only, 1-VI 2-4 branched and 1-VII single or double in about equal frequency.

The larva of *takasagoensis* is very similar to that of *dirus* and *balabacensis* and differs from both primarily in the length and placement of seta 4-C (Fig. 2), and from *balabacensis* in the weakly developed seta 1-II. The length of 4-C is shorter than that of either *dirus* or *balabacensis* and this, combined with the usually greater distance separating the insertions of 2-C and 4-C, gives it an even shorter appearance. The length of 4-C in *takasagoensis* is 0.044-0.072 mm with mean length of 0.062 mm. In *dirus* the length of 4-C is 0.062-0.091 mm with mean length of 0.077 mm. The distances between the insertions of 2-C and 4-C in *takasagoensis* and *dirus* are 0.084-0.111 mm ($\bar{X}$=0.100 mm) and 0.058-0.099 ($\bar{X}$=0.080 mm), respectively. The ratio of the length of 4-C to this distance is usually expressed as the distance 4-C extends forward divided by the distance between the insertions of 2-C and 4-C. In *takasagoensis* and *dirus* 4-C extends forward 0.46-0.76 ($\bar{X}$=0.63) and 0.69-1.29 ($\bar{X}$=0.97) the distance between the insertions of 2-C and 4-C, respectively. The length of 4-C and distance between the insertions of 2-C and 4-C in *balabacensis* is similar to that of *dirus*. In both *dirus* and *balabacensis* 4-C usually extends to very near or beyond the insertion of 2-C, while that of *takasagoensis* falls considerably short of the insertion of 2-C. Seta 4-C in *takasagoensis* is often branched at or near the middle, while the other 2 species often have 4-C bifurcated near the apex. The above measurements were made from mounted skins that were determined to be without tears or noticeable distortions, and with seta 4-C extended and close to one focal plane; even so, measurements should be viewed as approximations only.

BIONOMICS. Due to the apparently restricted distribution of *takasagoensis* on the island of Taiwan, this species has not received much attention. The limited published data on the bionomics of *takasagoensis* suggest habits typical of most members of the Leucosphyrus Group elsewhere in Southeast Asia.

The distribution of *takasagoensis* is most closely associated with foothill and mountainous regions of central and southern Taiwan. However, a specimen in our collection is labelled as having been collected in the area of Taipei. The favored immature habitat appears to be shaded rock pools, stream pools and pools in drying stream beds. Matumoto and Motoura (1939) collected larvae in "fairly dirty ponds." Morishita (1946), giving an account by Motoura, states "The breeding places were grassy buffalo hoof marks and pools in a Chomphor tree forest on a hill (189 m above sea level) at inland-side of coastal mountain range. The pools were approximately 10 cm deep, and were thought to have been used by buffaloes for bathing. The water was not spring water but simply stagnant and dirty." Collections of the larvae were made during the season from April to May [English translation of Morishita (1946) by J. C. Lien, 1961]. Chang and Huang (1954) report "It is a wild species found along foothills and occasionally in stables. The typical breeding place of this species is in heavily shaded rock pools and the like, in beds of mountain streams under jungle cover. However, it was recorded on several occasions from highly shaded pools or under sunny situations." J. C. Lein (*in litt.*) reports collecting the species, in the

Peiyuan, Tungho area throughout the year with a moderately high population density fluctuating between alternate months from October 1967 through July 1968. He stated that since the larvae develop most commonly in isolated pools in stream beds, they are very often flushed by heavy rain, and this was perhaps the main reason for the fluctuation in population density. He also stated that the feeding activity of the females, as determined by a man-bait-trap, started soon after sunset at around 1830 h and reached a peak at 2200 h, and then gradually diminished toward sunrise at around 0500 h in May to July. A similar pattern was obtained by using a monkey-bait-trap with the peak appearing at 2300 h.

MEDICAL SIGNIFICANCE. *Anopheles takasagoensis* has never been incriminated as a natural vector of malaria parasites and is usually reported as unimportant owing to its rarity. However, Chuang et al. (1965, 1967) reported high numbers of *takasagoensis* in Tungho township, Taitung county in southeastern Taiwan. The species was readily attracted to monkey-bait-traps. These authors found an exceptionally high incidence of *Plasmodium inui* Halberstaedter and von Prowazek, infections in 71.9 percent of the monkeys in this area. In a 3 year island-wide survey of malaria incidence in indigenous monkeys, they found a very close association between the occurrence of *P. inui* and *takasagoensis*, and concluded the following: "It now becomes clear that in Taiwan *P. inui* infections in monkeys are confined within the range of the distribution of *A. balabacensis takasagoensis*. The high incidence of *P. inui* infections in monkeys in Tungho township coincides with the high population of *Anopheles balabacensis takasagoensis* in the area. Although no concrete proof has been obtained, this mosquito is now suspected to be the vector of *P. inui* in Taiwan." According to J. C. Lien *in litt.* to R. A. Ward, *takasagoensis* in Tungho township, was "readily attracted to man and monkeys at a ratio of about 2-1." The highest catch, recorded in 4 nights during May 1968, in Tungho township, was 238 females atrracted to man on the ground and 132 to monkeys on a platform in the tree canopy about 7 meters above ground.

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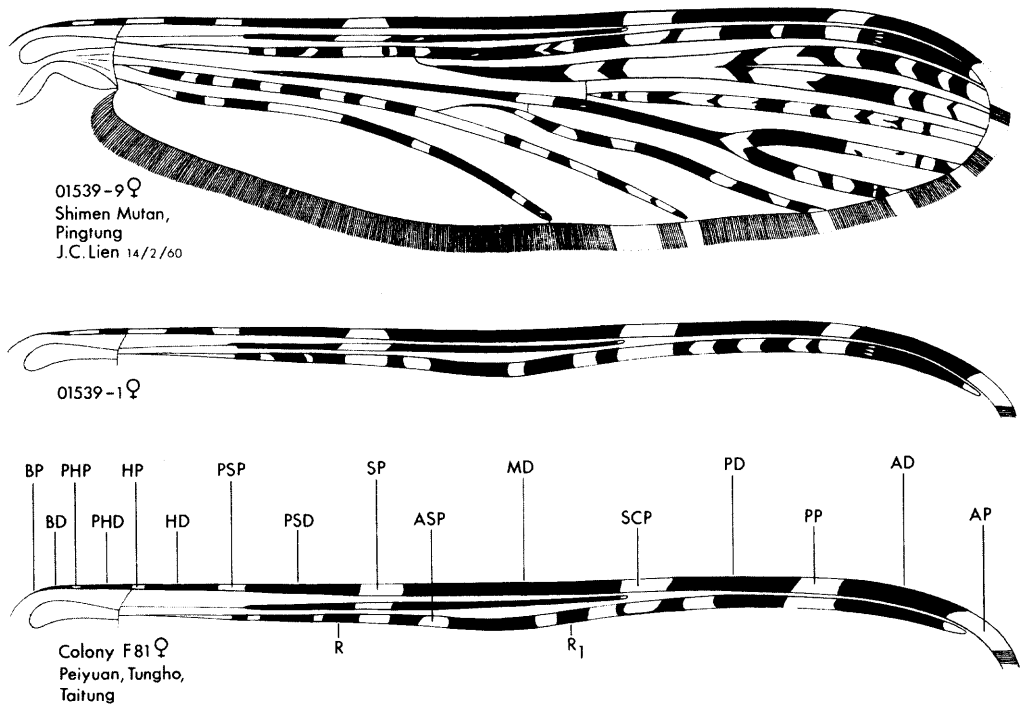
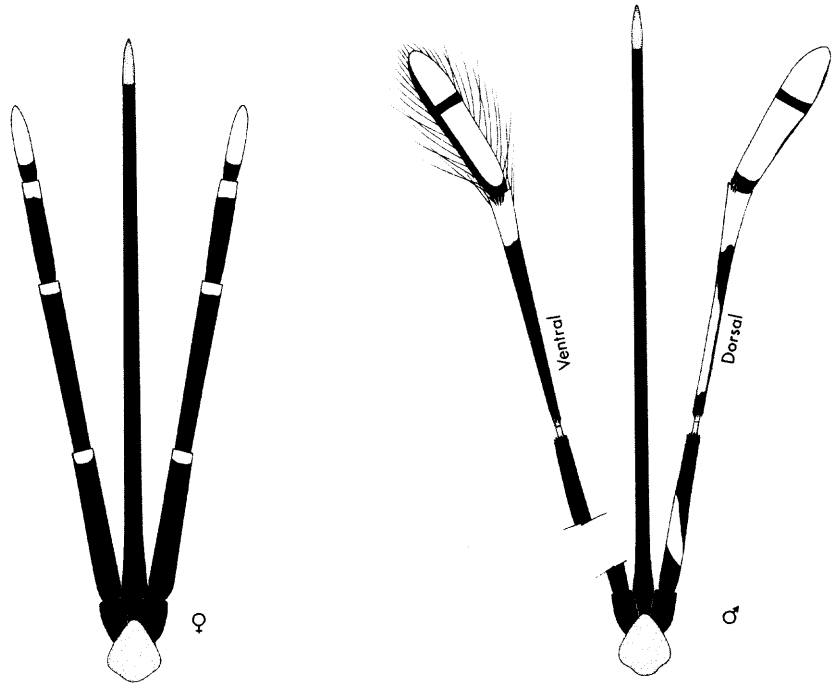
Fig.1

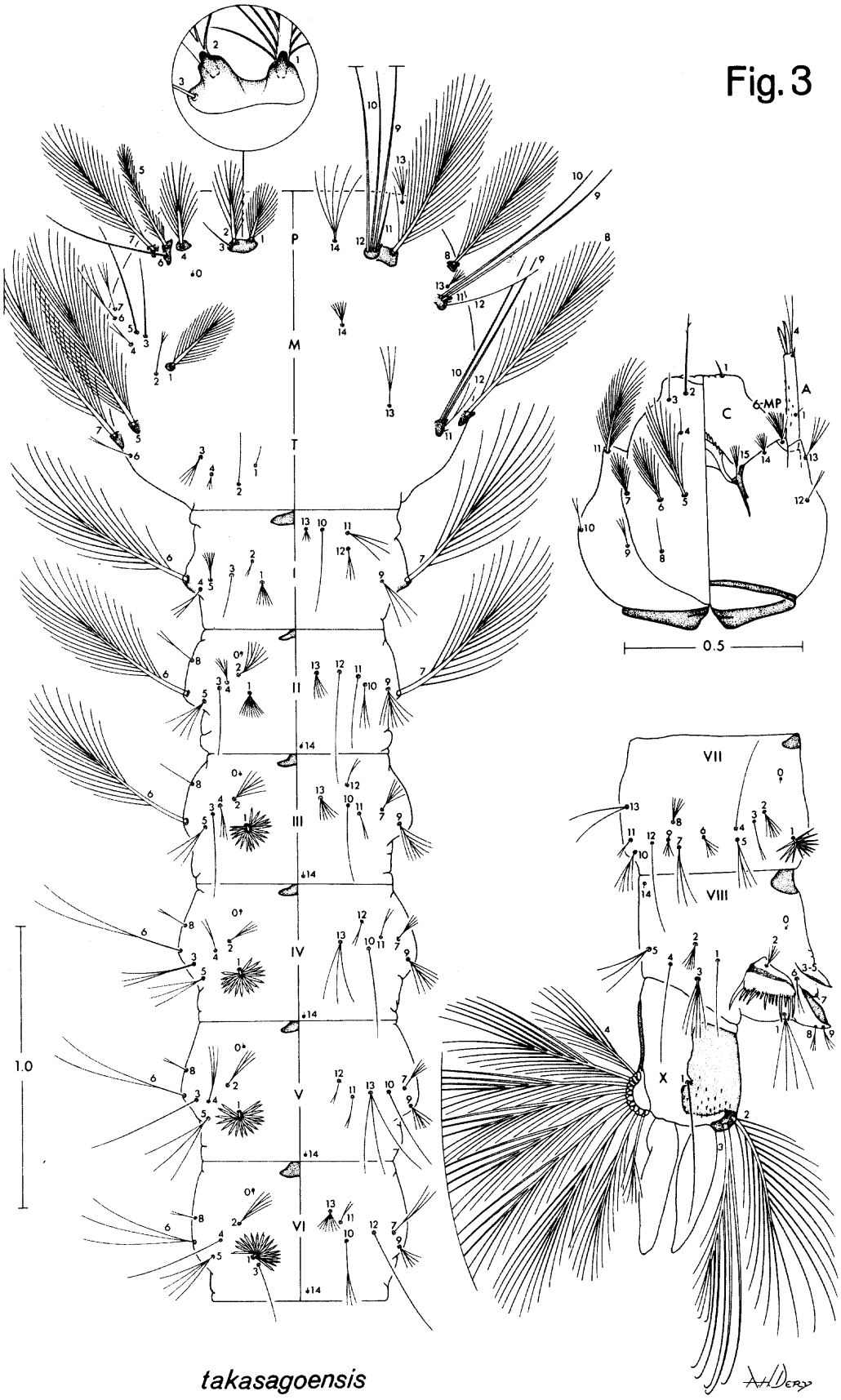




takasagoensis

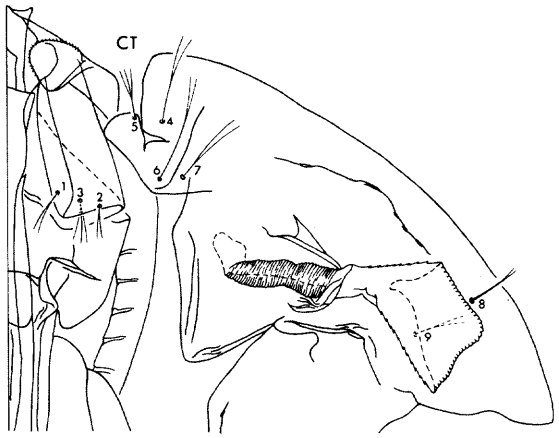
Vichai Malikul

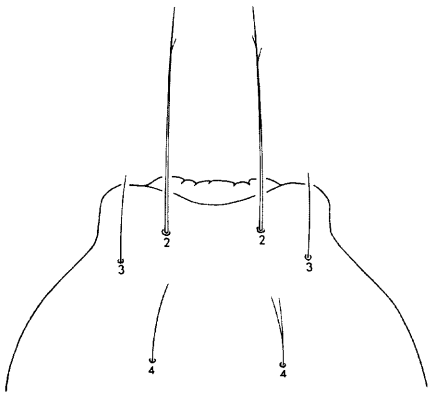
Fig. 3



takasagoensis

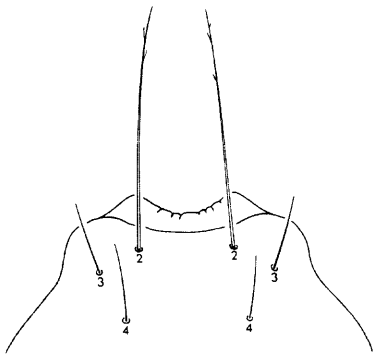
Fig. 2



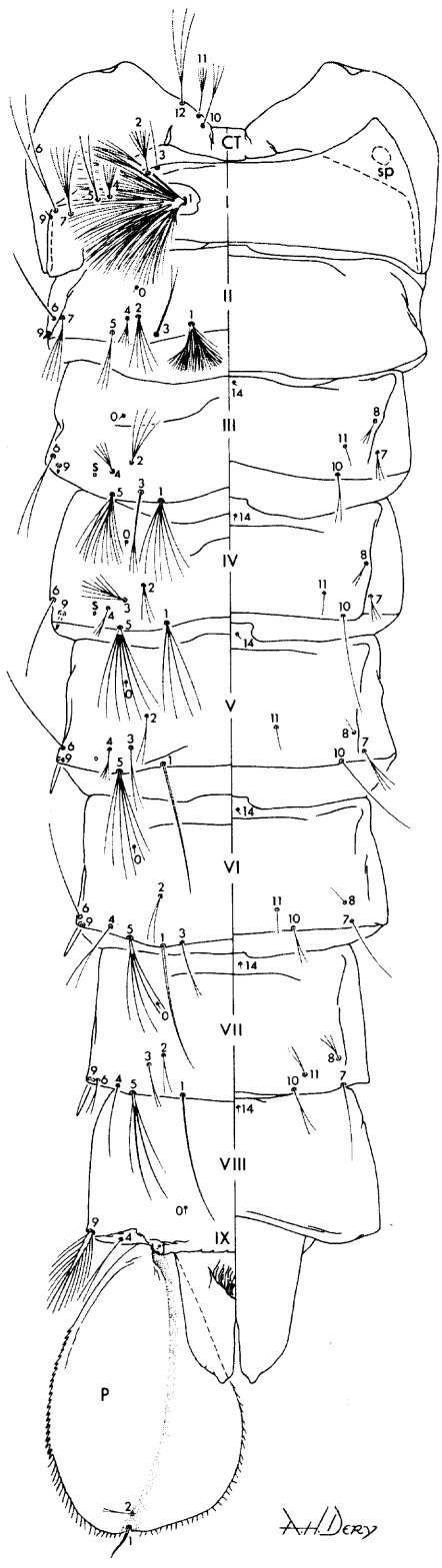


takasagoensis (Larval setae 2-4-C)
(Drawn from skins)

0.25



balabacensis and *dirus*
(Larval setae 2-4-C)



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